Remarks

In the Office Action dated July 8, 2002, the Examiner rejected claims 1-18 under 35 U.S.C. § 103 as being unpatentable over either of Trulson, et al. U.S. Patent No. 5,578,832 or Brown, et al. U.S. Patent No. 5,807,522 in view of the U.S. Patent to Ginestet U.S. Patent No. 6,225,636.

By this Amendment, Applicants' attorney has amended each of the independent claims to more particularly point out and distinctly claim what Applicants regard as their invention. In particular, independent claims 1 and 10 have been amended to make it clear that the set of correction factors are applied to quantitation data obtained from generated microarray images containing spots having three or more dyes with excitation or emission spectra to obtain cross-talk corrected data. Clearly, this feature is neither taught, disclosed or discussed by any of the prior art references of record taken either alone or in combination with one another as described below.

For example, Ginestet (The corresponding PCT application previously noted in the IDS of January 25, 2001) provides a reasonably comprehensive description on how to perform matrix math for crosstalk correction, but his context is FISH (Fluorescence In-Situ Hybridization) imaging of tissues. FISH utilizes fluorescent labeling chemistry similar to that used in microarrays, except the immobilized sample on the microscope slide is a thin section of tissue. The fluorescently-labeled DNA probe preferentially binds ("Hybridizes) to genes still located in the cells in the tissue (hence "In Site"), lighting up particular genes in the sample with a particular dye. As the sample is not a microarray, the fluorescent images that are acquired can have arbitrary geometry: they are just tissues with pseudo-random locations of cells. Therefore, the "color compensation" disclosed by Ginestet is done on a pixel-by-pixel basis throughout the image.

Attached here are two example FISH images, the same mouse kidney tissue imaged with two different fluorophores Cy3 and Cy2. These are small segments of larger images, but one can easily see how disordered they are compared to microarrays.

FISH images generally have a million or more pixels x 3 or more colors so the pixel-by-pixel "color compensation" described involves quite a bit of computing to apply.

The present invention as now claimed applies the crosstalk correction to quantitation data derived from microarray images, not to each pixel in the images themselves. As is known in the art, microarray image quantitation uses various algorithms to locate and isolate array spots comprised of many pixels and derive a single-number intensity value for each spot, for each color.

Quantitation reduces the data by at least two orders of magnitude, which is particularly significant since microarray images typically have many more pixels than FISH images: anywhere from 4 million to 12 million pixels per color is typical in microarrays. An example of data reduction by quantitation of a small single-color microarray is as follows: 20mm x 20mm image with 10micron pixels = 4 million pixels, each with 2-bytes of intensity = 8 Mbyte. This 8 Mbyte image would typically hold a microarray with 10,000 spots. The resulting quantitated data would thus be 10,000 spots x 2 bytes/spot = 20 kByte. 8 Mbyte / 20 kByte = factor of 400 data reduction by quantitation. Image data from larger microarrays would scale down by about the same factor.

In the present invention, correction is applied to the quantitation data, not to the image pixels. This approach can only work with arrays; one can't reduce a tissue image to a relatively short table of spot intensity values because of the random spatial features of tissue. This results in a significantly higher-performance microarray analysis system than if images were crosstalk-corrected on a pixel-by-pixel basis.

Also, Ginestet discloses performing the initial calibration by acquiring N images, where N is the number of dyes "if a calibration scene containing easily identifiable single-labeled chromosomes can be obtained. Such a scene would contain some chromosomes labeled with only dye F1, some labeled with only dye F2, etc., each easily identifiable by means other than their color." (column 11, line 33) This makes it sound easy, but the process does not appear to be enabled. How would the user know that a particular portion of a

particular tissue image has only one target "easily identified by means other than color"? The "scene" is just a tissue sample; one cannot be sure that any particular region contains one and only one binding partner to the mix of fluorescent probes.

The present invention uses calibration dye spots each of which is a single pure dye.

In the same vein, a FISH tissue-sample calibration "scene" is not reproducible, so the calibration process itself is not precisely reproducible.

Consequently, in view of the above and in the absence of better art, Applicants' attorney respectfully submits that the application is in condition for allowance which allowance is respectfully submitted.

Respectfully submitted,

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Attachment

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In The Claims

1. (Four Times Amended) A method for automatically creating crosstalk-corrected data of a microarray wherein crosstalk is caused by overlapping dye emission spectra, the method comprising:

providing a microarray substrate having three or more calibration dye spots, each of the calibration dye spots comprising a single pure dye;

for each of the calibration dye spots, generating a dye image containing at least one of the calibration dye spots for each of a plurality of output channels;

for each of the calibration dye spots, measuring an output of each of the output channels to obtain output measurements;

computing a set of correction factors from the output measurements; and

applying the set of correction factors to quantitation data obtained from the generated microarray images containing spots having three or more dyes with excitation or emission spectra to obtain crosstalk-corrected data.

10. (Four Times Amended) A system for automatically creating crosstalk-corrected data of a microarray wherein crosstalk is caused by overlapping dye emission spectra, the system comprising:

a microarray substrate having three or more calibration dye spots, each of the calibration dye spots comprising a single pure dye;

an imager having a plurality of output channels wherein for each of the calibration dye spots the imager generates a dye image containing at least one of the calibration dye spots for each of the output channels;

means for measuring an output of each of the output channels for each of the calibration dye spots to obtain output measurements;

means for computing a set of correction factors from the output measurements; and

means for applying the set of correction factors to quantitation data obtained from the generated microarray images containing spots having three or more dyes with excitation or emission spectra to obtain crosstalk-corrected data.